Hemicellulose Isolation from Annual Plants*

R. L. CUNNINGHAM, M. E. CARR, and M. O. BAGBY
Northern Regional Research Center,
Agricultural Research Service,
U.S. Department of Agriculture,†
Peoria, Illinois 61604

Summary

Wheat straw and sweet sorghum bagasse contain about 25% pentosans (hemicelluloses) and kenaf contains 26% in the core and 18% in the bark. Treatment of these milled materials with a 12% NaOH solution for 4 h at 80°C extracted 88–90% of the pentosans from wheat straw and sweet sorghum bagasse, and 80–84% from the kenaf bark and core. Ethanol precipitation of the filtrates recovered 90% of the extracted pentosans from wheat straw and sweet sorghum bagasse, and 66–76% of those extracted from the kenaf bark and core. Keywords: wheat straw, sweet sorghum bagasse, kenaf, pentosans, sodium hydroxide.

INTRODUCTION

Currently, few chemicals derived from hemicelluloses (pentosans) are of industrial importance. Xylose and furfural are the most prominent. These chemicals are produced from wastes: agricultural, wood, and spent pulping liquors. Following cellulose and lignin, hemicelluloses may be the third most abundant organic resource available.[1] Recently, there has been much interest in utilizing xylose, the main building block (or monomer) of hemicelluloses, as a substrate for fermentation. Production of 2, 3-butanediol, bioconversion to triglycerides by oil-rich yeasts, and ethanol production by *Pachysolen tannophilus* have been studied as possible products from hemicelluloses.[2–4] Chahal[5] has discussed the use of hemicelluloses as energy sources for fermentation: *Trichoderma reesei* for enzyme production, and *Chaetomium cellulolyticum* and *Pleurotus sajor-caju* for single cell protein.

Earlier, we evaluated sodium hypochlorite, sodium hydroxide, and sulfuric acid, either alone or in selected combinations and sequences, to extract pentosans from wheat straw.[6] One of the most effective methods was extraction of milled wheat straw with a 12% NaOH solution. Because both monocotyledonous and dicotyledonous plants contain high quantities of hemicelluloses, we examined

* Presented at the Eighth Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, Tennessee, May 13–16, 1986.

† The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Biotechnology and Bioengineering Symp. No. 17 (1986)
 CCC0572-6565/87/170159-10$04.00

two crop residues, wheat straw and sweet sorghum bagasse (monocots), and a prolific biomass source, kenaf (a dicot), as potential sources of hemicelluloses. With the advances in biotechnology, breakthroughs may permit new agribusiness to rival existing business of the chemical industry. Therefore, the capability of efficiently separating and recovering this very abundant but little-used resource, hemicellulose, may permit it to become a significant segment of future agribusiness.

MATERIALS AND METHODS

Baled straw of soft winter wheat, *Triticum aestivum* L., variety Arthur, from Central Illinois was cut into 2- to 8-cm lengths in a Taylor, Stiles, and Company chopper. Sweet sorghum, *Sorghum bicolor* (L.) Moench, variety Wray, grown in Central Illinois was harvested at 140 days. Stalks were stripped of leaves and culms were sectioned. Culms were dejuiced by pressing in a cylindrical mold (2-¼-inch diameter) with a hydraulic press and samples of both culms and bagasse were freeze dried. Kenaf, *Hibiscus cannabinus* L., grown in Maryland was separated into bark and core components and cut into sections. All samples were ground in a Wiley-type mill equipped with a screen containing 1 mm-diameter openings.

Alkali Extraction

Ten-gram samples (moisture-free basis) of ground plant material were mixed with 100 mL of 10, 12, 14, or 16% NaOH (w/v) and steeped 4 h at either 80 or 99°C. Residues obtained after filtering were washed with 400 mL of hot water. Some filtrates were acidified to pH 4.5 with acetic acid, and others were left alkaline. Then, ethanol (95%) was added to each, providing an ethanol:filtrate ratio of 2:1. After standing 24 h, the precipitates formed in the alcoholic media were collected on filter paper, washed with absolute ethanol, air dried, and then analyzed. The initial alkali-insoluble residues were water washed again to remove residual alkali, filtered, and then freeze dried.

Cellulase Treatments and Chemical Analyses

To assess cellulose availability, cellulosic materials were treated with cellulase by a procedure previously reported.[7] Starting materials were air dried (wheat straw and kenaf) or freeze dried (sweet sorghum bagasse), but alkali-insoluble residues were, when necessary, stored frozen but never dried. Glucose yields were determined by high-performance liquid chromatography (HPLC) with a Bio Rad (Richmond, CA) HPX-87H size exclusion column and water as the mobile phase. Cellulose contents were measured by a monoethanolamine method[8] and reported on an ash-free and pentosan-free basis. An ultraviolet spectrophotometric method[9] was used for lignin analysis. Samples were analyzed for pentosan contents by TAPPI Standard Method T223m and for ash contents by

ignition at 600 ± 25°C. Values reported are means of duplicate analyses determined to be within ±2%.

RESULTS AND DISCUSSION

Plant Composition

Many potential renewable sources of carbohydrates for energy have been identified.[10] Ethanol or other fermentation products generated from the carbohydrates could be major feedstocks for the synthesis of a wide range of polymers and lower molecular weight chemicals. The chemical composition of the three high-yielding sources of carbohydrates considered in this study (wheat straw, sweet sorghum, and kenaf), are reported in Table I. Even though sweet sorghum is lower in cellulose and pentosans, considerable sugar (mainly sucrose), evinced by the ethanol-soluble portion, was present in the stalks. Juice, containing 14% sugar, has been obtained in 50 to 68% yields and can be fermented readily by *Saccharomyces cerevisiae* to ethanol in 72% yield.[11]

Wheat Straw

Sloneker[12] has indicated that harvested grain represents less than half the solar energy captured by cultivated plants. The plant components left in the fields after harvesting account for the remainder. When a major crop such as wheat produces 1–2 tons/acre of residue, conversion of a portion of this quantity of organic matter to useful products could contribute greatly to agriculture's profitability. Isolation of major constituents is one means of upgrading the value of such residue. Steeping ground wheat straw with 10 or 12% NaOH solution for 4 h at

TABLE I
Potential Sources of Hemicelluloses

Plant classification	Monocotyledonous		Dicotyledonous
Plant	*Triticum aestivum* (wheat straw)	*Sorghum bicolor*[a] (sweet sorghum)	*Hibiscus cannabinus* (kenaf)
Component (%)			
Cellulose, MEA[b]	30	16 (27)	39
Pentosans	25	16 (24)	23
Lignin	18	6 (9)	10
Solubles[c]	8	59 (33)	4

[a] Values in parentheses represent composition for sweet sorghum bagasse.
[b] Monoethanolamine method.[8]
[c] Values represent solubility in alcohol-benzene except for sweet sorghum which is solubility in 80% ethanol.

TABLE II

Characteristics of Alkali-Extracted Wheat Straw[a]

Analyses (%)	Treatment		
		Concentration of NaOH (%)	
	Untreated	10	12
Yield[b]	—	35.8	34.7
Solubility in alcohol–benzene	8.1	1.5	1.4
Cellulose, MEA[c]	30.3	79.2	79.5
Pentosans	25.0	8.0	7.5
Lignin	18.0	8.5	8.6
Ash	11.3	3.0	2.5
Cellulose conversion	21.3	83.7	86.0
		Precipitated liquor solids[d]	
Yield[b]		41.0	43.4
Pentosans		48.2	46.3
Lignin		5.5	6.2
Ash		37.0	38.5

[a] NaOH solution: straw (<1 mm), 10:1; 4 h at 80°C.
[b] (Insoluble fraction weight ÷ sample weight) × 100.
[c] Monoethanolamine method.[8]
[d] Solids precipitated by addition of ethanol to filtrates.

80°C extracted 89–90% of the pentosans and 83% of the lignin (Table II). The computation for determining percentage of extraction was as follows:

$$\frac{\%\text{ component, untreated} - (\%\text{ yield, treated} \times \%\text{ component, treated})}{\%\text{ component, untreated}} = \%\text{ extracted component}$$

The % yield in the equation represents the weight of the insoluble fraction recovered divided by the weight of the untreated sample × 100. Cellulase treatments of unextracted wheat straw converted 21% of its cellulose to glucose, whereas a fourfold improvement occurred in the susceptibility of the cellulose to enzymatic hydrolysis after alkali extraction.

When ethanol was added to the NaOH extractives, 89–90% of the extracted pentosans were recovered. The computation for determining percentage of extracted pentosans recovered was as follows:

$$\frac{\%\text{ yield, precipitate} \times \%\text{ pentosans, precipitate}}{\%\text{ pentosans, untreated} - (\%\text{ yield, treated} \times \%\text{ pentosans, treated})} = \%\text{ extracted pentosans recovered}$$

This represents a slight improvement over the 85% pentosans recovered by acidified alcohol precipitation reported earlier.[6] The ethanol-precipitated liquor solids have high mineral contents (37–38%). It is known in the pulping industry

that liquors from alkaline pulping contain carbohydrates as sodium salts and have been degraded in terms of molecular weight to the extent that they are not precipitated by acid.[13] Although our alkali treatments were not performed under the high temperatures and pressures associated with alkaline pulping, some of the breakdown reactions may still have taken place. Little is known about these complex reactions. Even though hemicellulose A and hemicellulose B can be isolated separately by pH adjustment prior to ethanol addition,[14] recovery of the total pentosans without the traditional neutralization or acidification has the advantage that both NaOH and ethanol can be recycled. Hemicellulose A is the fraction that precipitates by the neutralization of the alkaline extract and the hemicellulose B fraction remains dissolved until precipitated with ethanol.

Sweet Sorghum Bagasse

Sweet sorghum has promise as a sugar crop for fuels.[15] It is more widely adapted to the climatic conditions of this country than sugar cane. Composition for sorghum bagasse is reported in Table III. When bagasse was treated with 12% NaOH solution for 4 h at 80°C, 88% of the bagasse pentosans and 83% of the lignin were extracted. These yields are similar to those obtained from the wheat straw. The susceptibility of the bagasse to enzymatic hydrolysis was increased from 32 to 81% by this NaOH treatment. Eighty-eight percent of the extracted pentosans were recovered by acidified alcohol precipitation. Ninety percent of the extracted pentosans were recovered when only ethanol was used for the precipi-

TABLE III

Composition and Properties of Alkali-Extracted Sweet Sorghum Bagasse[a]

	Treatment	
Analyses (%)	Untreated	12% NaOH
Yield[b]	—	32.6
Solubility in 80% ethanol	33.1	1.6
Cellulose, MEA[c]	27.2	83.2
Pentosans	23.9	8.8
Lignin	9.1	4.7
Ash	2.3	1.3
Cellulose conversion	32.5	81.3
		Precipitated liquor solids[d]
Yield[b]		27.3 (28.0)
Pentosans		67.9 (67.4)
Lignin		6.0 (4.5)
Ash		15.2 (24.3)

[a] NaOH solution: bagasse (<1 mm), 10:1; 4 h at 80°C.

[b] (Insoluble fraction weight ÷ sample weight) × 100.

[c] Monoethanolamine method.[x]

[d] Solids precipitated by addition of acetic acid and ethanol to filtrates. Values in parentheses represent solids precipitated by addition of ethanol alone to filtrates.

tation. However, 22% of the extracted lignin was recovered by acidified alcohol precipitation compared to only 17% by ethanol precipitation. A higher mineral content was observed in the ethanol precipitate than in the acidified alcohol precipitate (24% versus 15%). The lower ash content in the acidified alcohol precipitate may be advantageous during purification for some uses.

Kenaf

Kenaf has been identified as a promising fiber crop.[16] This annual plant is a dicotyledon and contains two types of fiber, bark and core. Their pulp characteristics have been described.[17] When kenaf stalks are chopped, the bark and core tend to separate, especially when dry. Samples separated into bark and core components for this study contained 36% bark and 64% core. Atchison and Collins[18] reviewed the worldwide use of kenaf, including whole stalk utilization as well as products derived from the fractionated bark and core. Residue yields from the bark and core were similar after steeping with 12% NaOH solution for 4 h at 80°C, but the composition of the residues were different except for pentosan contents (Table IV). Cellulose content of the bark residue was 72% versus 60% for that of the core. Lignin content of bark residue was 10% compared to 22% for the core residue. The reason for the higher residual lignin contents in the kenaf residues compared to the wheat straw and sweet sorghum bagasse residues

TABLE IV

Characteristics of Alkali-Extracted Kenaf Components[a]

	Treatment			
	Untreated		12% NaOH	
Analyses (%)	Bark	Core	Bark	Core
Yield[b]	—	—	59.6	58.5
Solubility in alcohol-benzene	3.8	4.6	1.1	1.0
Cellulose, MEA[c]	45.1	36.5	71.9	59.8
Pentosans	17.8	25.6	5.9	6.8
Lignin	7.9	13.6	10.1	21.9
Ash	6.3	2.8	3.5	1.9
Cellulose conversion	2.5	0.0	68.9	69.4
			Precipitated liquor solids[d]	
Yield[b]			12.3 (17.8)	21.2 (23.1)
Pentosans			68.2 (53.0)	76.2 (70.7)
Lignin			7.4 (6.6)	2.6 (4.2)
Ash			11.8 (30.8)	19.9 (25.8)

[a] NaOH solution:kenaf (<1 mm), 10:1; 4 h at 80°C.

[b] (Insoluble fraction weight ÷ sample weight) × 100.

[c] Monoethanolamine method.[8]

[d] Solids precipitated by addition of acetic acid and ethanol to filtrates. Values in parentheses represent solids precipitated by addition of ethanol to filtrates.

may be due to the fact that kenaf has a lignin structurally like hardwood lignin, whereas wheat straw and sweet sorghum have a typical grass-type lignin.[19] Pentosan contents of the residues were similar at 6–7%. This represents an 80% extraction of the bark pentosans and 84% extraction of the core pentosans. Twenty-four percent of the kenaf bark lignin was removed but only 6% of the kenaf core lignin. Only 59% of the pentosans extracted from the bark were recovered by acidified alcohol precipitation and 66% by ethanol precipitation. Of the pentosans removed from the core, 75–76% of extracted pentosans were recovered by either the acidified alcohol or ethanol precipitation method. These recovery differences are consistent with hemicellulose data, previously unpublished, for similar kenaf materials grown in Florida and Illinois (Table V).[20] The relative amounts of hemicelluloses A and B were similar for the bark. However, for the core samples, the quantity of hemicellulose A was nearly double that of the more soluble hemicellulose B.

The effect of increasing the concentration of NaOH in treating kenaf core is reported in Table VI. Only 2% more of the core pentosans were removed by increasing the concentration of the NaOH solution from 12 to 14 or 16%. Ethanol precipitation permitted the recovery of 70–78% of those pentosans removed. These lower values may again reflect the heterogeneity differences in pentosans from monocots and dicots. With 12% NaOH solution, 6% of the kenaf core lignin was extracted. Stronger concentrations of NaOH extracted additional lignin from the kenaf core.

Results of increasing the reaction temperature from 80 to 99°C in treating kenaf core with 14% NaOH solution are reported in Table VII. An additional 2%

TABLE V

Composition of Hemicelluloses Extracted from Fractionated Kenaf[a]

	Fraction			
	Bark		Core minus pith	
	Hemicellulose		Hemicellulose	
Analyses (%)	A	B	A	B
Yield[b]	7.6	7.9	14.7	7.5
Composition, GLC[c]				
Xylose	97.3	94.4	84.2	87.6
Arabinose	1.2	1.8	0.3	0.6
Ribose	0.4	0.6	0.5	ND[d]
Galactose	0.2	1.2	ND	0.2
Glucose	0.1	0.8	12.0	10.1
Rhamnose	0.6	0.8	1.3	1.4
Fucose	0.2	0.4	1.7	0.1

[a] M. O. Bagby.[20]

[b] (Insoluble fraction weight ÷ sample weight) × 100.

[c] Neutral sugars, GLC = gas liquid chromatography.

[d] ND = not detected.

TABLE VI

Characteristics of Alkali-Extracted Kenaf Core[a]

Analyses (%)	Concentration of NaOH (%)		
	12	14	16
Yield[b]	58.5	57.9	57.9
Solubility in alcohol-benzene	1.0	0.8	1.0
Cellulose, MEA[c]	59.8	62.6	62.6
Pentosans	6.8	5.9	6.0
Lignin	21.9	18.1	20.4
Ash	1.9	1.8	1.9
Cellulose conversion	69.4	70.3	67.7
	Precipitated liquor solids[d]		
Yield[b]	23.1	22.9	28.8
Pentosans	70.7	67.6	59.7
Lignin	4.2	2.0	1.2
Ash	25.8	34.4	36.5

[a] NaOH solution:kenaf core (<1 mm), 10:1; 4 h at 80°C.
[b] (Insoluble fraction weight ÷ sample weight) × 100.
[c] Monoethanolamine method.[8]
[d] Solids precipitated by addition of ethanol to filtrates.

TABLE VII

Effect of Temperature on Extracting Kenaf Core with 14% NaOH Solution[a]

Analyses (%)	Temperature (°C)	
	80	99
Yield[b]	57.9	55.3
Solubility in alcohol-benzene	0.8	0.8
Cellulose, MEA[c]	62.6	64.5
Pentosans	5.9	5.2
Lignin	18.1	18.7
Ash	1.8	2.0
Cellulose conversion	70.3	71.4
	Precipitated liquor solids[d]	
Yield[b]	22.9	25.2
Pentosans	67.6	62.2
Lignin	2.0	5.7
Ash	34.4	36.7

[a] NaOH solution:kenaf core (<1 mm), 10:1; 4 h.
[b] (Insoluble fraction weight ÷ sample weight) × 100.
[c] Monoethanolamine method.[8]
[d] Solids precipitated by addition of ethanol to filtrates.

pentosans (basis, kenaf core pentosans) were extracted at the higher temperature. Twenty-four percent of the core lignin was extracted at 99°C and 23% at 80°C. Although 89% of the core pentosans were extracted at the higher temperature, only 69% were recoverable by ethanol precipitation from the extract.

Extractability and Recovery of Pentosans

A comparison of the pentosan contents of wheat straw, sweet sorghum bagasse, and kenaf before and after treatment with a 12% NaOH solution for 4 h at 80°C is made in Figure 1. These data show that less pentosans (basis residue) remained in the monocotyledonous plant residues than in the dicotyledonous plant, kenaf. Likewise, more pentosans were recovered by ethanol precipitation from the alkaline extracts of the monocotyledonous plants than from kenaf.

CONCLUSIONS

Treatment of wheat straw and sweet sorghum bagasse with a 12% NaOH solution for 4 h at 80°C extracted 88–90% of the available pentosans from these plant materials. An ethanol precipitation of the alkaline extracts without neutralizing recovered pentosans equivalent to 19–20% of the dry weight of the plant

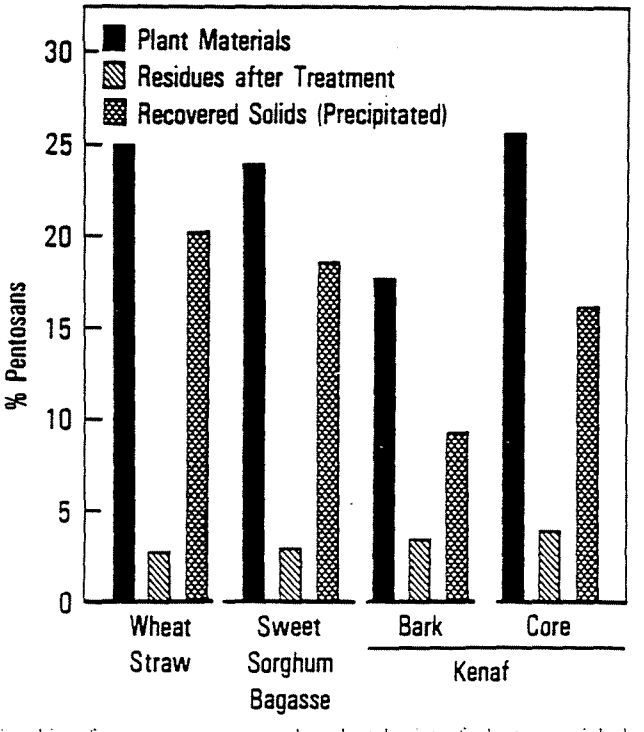

Fig. 1. Relationship of pentosan contents (dry plant basis) of plant materials before and after treatment [12% NaOH solution:plant material (<1 mm), 10:1; 4 h at 80°C]. Pentosans (dry plant basis) recovered from filtrates by precipitation with ethanol.

materials. This same process permitted 9% of the kenaf bark and 16% of the kenaf core to be recovered as pentosans. The monocots, wheat straw and sweet sorghum bagasse, responded well to this process and could be an excellent source of pentosans for industrial raw materials.

The authors thank D. W. Ehmke and D. M. Palmer for their technical assistance and Dr. K. D. Carlson for providing us with sweet sorghum bagasse.

References

[1] N. S. Thompson, "Chemicals from Hemicelluloses," in *Organic Chemicals from Biomass*, I. S. Goldstein, Ed. (CRC Press, Inc., Boca Raton, FL, 1981), p. 127.

[2] E. K. C. Yu, L. Deschatelets, and J. N. Saddler, *Appl. Microbiol. Biotechnol.*, **19**, 365 (1984).

[3] R. Fall, P. Phelps and D. Spindler, *Appl. Environ. Microbiol.*, **47**(5), 1130 (1984).

[4] R. W. Detroy, R. L. Cunningham, and A. I. Herman, *Biotechnol. Bioeng. Symp.*, **12**, 81 (1982).

[5] D. S. Chahal, *Biotechnol. Bioeng. Symp.*, **14**, 425 (1984).

[6] R. L. Cunningham, M. E. Carr, and M. O. Bagby, *Biotechnol. Bioeng. Symp.*, **15**, 17 (1985).

[7] R. W. Detroy, L. A. Lindenfelser, G. St. Julian, Jr., and W. L. Orton, *Biotechnol. Bioeng. Symp.*, **10**, 135 (1980).

[8] G. H. Nelson and J. A. Leming, *Tappi*, **40**(10), 846 (1957).

[9] M. O. Bagby, R. L. Cunningham, and R. L. Maloney, *Tappi*, **56**(4) 162 (1973).

[10] J. Coombs, *Outlook on Agriculture*, **10**(5), 235 (1980).

[11] K. D. Carlson, R. L. Cunningham, and A. I. Herman, *Trans. Ill. State Acad. Sci.*, **76**(1, 2), 111 (1983).

[12] J. H. Sloneker, *Biotechnol. Bioeng. Symp.*, **6**, 235 (1976).

[13] G. E. Jackson, "Alkaline Pulping," in *Handbook of Pulp and Paper Technology*, K. W. Britt, Ed. (Reinhold Publishing Corporation, New York, 1964), p. 173.

[14] R. L. Whistler and M. S. Feather, "Hemicellulose-Extraction from Annual Plants with Alkaline Solutions," in *Methods in Carbohydrate Chemistry* V. R. L. Whistler, Ed. (Academic Press, New York, 1965), pp. 144-145.

[15] R. A. Nathan, Ed., *Fuels from Sugar Crops* (USDOE Technical Information Center, Oak Ridge, TN, 1978).

[16] M. O. Bagby, in *Non-Wood Plant Fiber Pulping Progress Report No. 8* (Technical Association of the Pulp and Paper Industry, Atlanta, GA, 1977), pp. 75-80.

[17] T. F. Clark, M. O. Bagby, R. L. Cunningham, G. F. Touzinsky, and W. H. Tallent, in *Non-Wood Plant Fiber Pulping Progress Report No. 2* (Technical Association of the Pulp and Paper Industry, Atlanta, GA, 1971), pp. 267-278.

[18] J. E. Atchison and T. T. Collins, Jr., in *Non-Wood Plant Fiber Pulping Progress Report No. 7* (Technical Association of the Pulp and Paper Industry, Atlanta, GA, 1976), pp. 15-27.

[19] T. P. Abbott and C. James, *Tappi*, **67**(4), 132 (1984).

[20] M. O. Bagby, unpublished results.